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Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

COMPANY SANITIZED

RE:

To whom it may concern:

is submitting this information pursuant to Section 8(e) of TSCA.

On November 20, 2001, submitted draft summary information concerning two toxicology studies with respect to , and claimed the chemical composition and company identity as Confidential Business Information (CBI). Attached are the final reports.

As previously indicated, the chemical composition and company identity is being claimed as CBI. The rationale for this claim is as follows: 1) the complete disclosure of the chemical substance identity along with the company name has never been made available to our competitors and 2) revealing this information would lead to a significant competitive disadvantage to Additional CBI justification is presented in Attachment 1. The current chemical represents one individual and unique substance. This CBI strategy will allow our company to protect sensitive information while giving the Agency and the public information about the chemical nature of the substance that is the subject of this submission.

The substance, is being used as an additive in an electrodeposition corrosion resistant primer for the Automotive OEM market sector. Application of the primer takes place in a large enclosed tank, which also contains other components such as resins, pigments, and various performance additives common to this coating process. Metal parts are transported by conveyer and dipped into the tank with electrical potential applied. The coating is deposited on the part as a wet film. Coated parts are rinsed and transported to a zone of elevated temperature to cure the wet film.

The rinse material is recycled back into the process as a closed system making accidental release highly unlikely. Solid residues from process treatment operations are disposed of in hazardous waste (RCRA Subtitle C regulated) landfills or incinerators or within a RCRA Subtitle D regulated landfill. It is estimated that there is no discharge of material to local POTW.

provides our customers with labeling and MSDS, which specify procedures for proper handling and disposal of products containing the chemical substance including the use of personal protective equipment.

Please telephone me at if you have any questions.

Attachments

Attachment No. 1

Substantiation of Confidentiality

1. Is your company asserting this confidential business information (CBI) claim on its own behalf? If the answer is no, please provide company name, address and telephone number of entity asserting claim.

   Yes, we are asserting this claim on our own behalf.

2. For what period do you assert your claim(s) of confidentiality? If the claim is to extend until a certain event or point in time, please indicate that event or time period. Explain why such information should remain confidential until such point.

   Confidential treatment should be maintained indefinitely in order to protect our company's know-how in this area.

3. Has the information that you are claiming as confidential been disclosed to any other governmental agency or to this Agency at any other time? Identify the Agency to which the information was disclosed and provide the date and circumstances of the same. Was the disclosure accompanied by a claim of confidentiality? If yes, attach a copy of said document reflecting the confidentiality agreement.

   No.

4. Briefly describe any physical or procedural restrictions within your company relating to the use and storage of the information you are claiming CBI.

   Special precautions taken to protect the confidentiality of this information include: the contracting of all employees to maintain confidentiality of all phases of their company activities; the maintaining of restricted entry facilities; the escort of non-company personnel within the facilities; and the dissemination of information on chemical composition on a need-to-know basis.

5. If anyone outside your company has access to any of the information claimed CBI, are they restricted by confidentiality agreement(s)? If so, explain the content of the agreement(s).

   The information has been disclosed to the toxicological testing laboratory under the terms of a confidentiality agreement. There has been no public disclosures or disclosures to competitors of the information, and there will not be such disclosures in the future.

6. Does the information claimed as confidential appear or is it referred to in any of the following:

   a. Advertising or promotional material for the chemical substance or the resulting end product;

   b. Material safety data sheets or other similar materials (such as technical data sheets) for the substance or resulting end product (include copies of this information as it appears when accompanying the substance and/or product at the time of transfer or sale);

   c. Professional or trade publications; or

   d. Any other media or publications available to the public or to your competitors.

   If you answered yes to any of the above, indicate where the information appears, include copies, and explain why it should nonetheless be treated as confidential.

   The claimed confidential information does not appear in any of the above references.

7. Has EPA, another federal agency, or court made any confidentiality determination regarding information associated with this substance? If so, provide copies of such determinations.

   There have been no confidentiality determinations made.

8. Describe the substantial harmful effects that would result to your competitive position if the CBI information is made available to the public? In your answer, explain the causal relationship between disclosure and any resulting substantial harmful effects. Consider in your answer such constraints as capital and marketing cost, specialized technical expertise, or unusual processes and your competitors' access to your customers. Address each piece of information claimed CBI separately.

   Yes, disclosure of this information would likely result in substantial harm to our company's competitive position in the marketplace. Normally, the first company in the marketplace with a new product is able to capture a fairly large market share. Disclosure of this confidential information would place our company at an economic disadvantage with our competitors. Our company has vigorously protected this information (chemical composition, company-name) under applicable laws regarding trade secrets.

9. Has the substance been patented in the U.S. or elsewhere? Is a patent for the substance currently pending?

   We are currently pursuing a patent.

10. Is this substance/product commercially available and if so, for how long has it been available on the commercial market?

The substance is commercially produced by and for our company only. We continue to evaluate various products with it.

a. If on the commercial market, are your competitors aware that the substance is commercially available in the U.S.?

N/A

b. If not already commercially available, describe what stage of research and development (R&D) the substance is in, and estimate how soon a market will be established.

The substance is undergoing further developmental testing.

c. What is the substance used for and what type of product(s) does it appear in.

A cationic electrodeposition corrosion resistant primer for the Automotive OEM market sector.

11. Describe whether a competitor could employ reverse engineering to identically recreate the substance?

It is possible that this could happen.

12. Do you assert that disclosure of this information you are claiming CBI would reveal:

a. confidential processes used in manufacturing the substance;

b. if a mixture, the actual portions of the substance in the mixture; or

c. information unrelated to the effects of the substance on human health or the environment?

If your answer to any of the above questions is yes, explain how such information would be revealed.

Disclosure of the information claimed as confidential would reveal information unrelated to the effects on human health or the environment. The information would reveal composition information and link that information to a specific chemical substance. Since the material tested was a PMN notification, releasing the exact composition would place our company at a competitive disadvantage. Further, the generic name is sufficient to interpret the study data presented.

13. Provide the Chemical Abstract Service Registry Number for the product, if known. Is your company applying for a CAS number now or in the near future? If you have applied for a CAS number, include a copy of the contract with CAS.

SafePharm Laboratories

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SPL PROJECT NUMBER: 1014/135

AUTHOR: P Brunt

STUDY SPONSOR:

TEST FACILITY:

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P.O. Box No. 45
DERBY
DE1 2BT
U.K.

Telephone: (01332) 792896

Facsimile: (01332) 799018

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	09 March 2001	Standard Test Method Compliance Audit
	11 September 2001	Test Material Preparation
	11 September 2001	Animal Preparation
	04 September 2001	Dosing
	14 September 2001	Assessment of Response
§	18 October 2001	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 03 DEC 2001

For Safepharm Quality Assurance Unit*

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*Authorised QA Signatures:

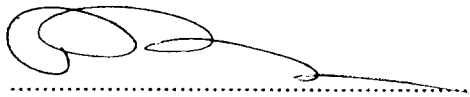
Head of Department: JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department: JM Crowther MISeT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA; R Hurst MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.



..... DATE: 23 NOV 2001

P Brunt HNC
Study Director

CONTENTS

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

SUMMARY

Introduction. The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
- Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)
- United States Environmental Protection Agency Health Effects Test Guidelines OPPTS 870.2600 Skin Sensitisation August 1998
- Japanese Ministry of Health and Welfare, 1992

Method. Twenty test and ten control animals were used for the study. Two phases were involved in the main study; an induction of a response by intradermal injection and topical application and a topical challenge of that response.

Based on the results of sighting tests, the concentrations of test material for the induction and challenge phases were selected as:

Intradermal Induction	:	1% v/v in arachis oil BP
Topical Induction	:	10% v/v in arachis oil BP
Topical Challenge	:	2% and 1% v/v in arachis oil BP

A topical rechallenge was performed at concentrations of 2% and 1% v/v in arachis oil BP.

Conclusion. Under the conditions of the test, the test material produced a positive response in 21% (4/19) animals following topical challenge at a concentration of 2% v/v in arachis oil BP. At topical rechallenge a positive response was noted in 42% (8/19) animals.

SKIN SENSITISATION IN THE GUINEA PIG - MAGNUSSON AND KLIGMAN MAXIMISATION METHOD

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992)
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- United States Environmental Protection Agency Health Effects Test Guidelines OPPTS 870.2600 Skin Sensitisation August 1998
- Japanese Ministry of Health and Welfare, 1992

The albino guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix 8). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 15 August 2001 and 11 October 2001.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	
Chemical name	:	
CAS number	:	
% Solid	:	97.8% (2.1% Toluene, 0.1% water)
Description	:	yellow coloured liquid
Lot Number	:	03817
Date received	:	30 April 2001
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of this study the test material was used undiluted and freshly prepared in arachis oil BP. The concentrations used are discussed in the procedure section.

The absorption of the test material was not determined.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Forty-eight male albino Dunkin Hartley guinea pigs were supplied by David Hall Limited, Burton-on-Trent, Staffordshire, UK. After an acclimatisation period of at least five days, each animal was selected at random and given a number unique within the study which was written on a small area of clipped rump using a black indelible marker-pen. At the start of the main study the animals were in the weight range of 374 to 490g, and were approximately eight to twelve weeks old. The bodyweight of one animal was above the weight specified in the Standard Test Method (450g). This was considered not to affect the purpose or integrity of the study.

The animals were housed singly or in pairs in solid-floor polypropylene cages furnished with woodflakes. Free access to mains tap water and food (Certified Guinea Pig Diet (Code 5026) supplied by PMI Nutrition International, Nottingham, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment, irradiated hay (Harlan UK Ltd, Bicester, Oxford), which was considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, J. Invest. Dermatol. (1969) 52: 268 - 276.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Intradermal Induction

Intradermal injections (0.1 ml/injection site) were made on the clipped shoulder of two guinea pigs, using concentrations of 1% and 5% v/v in arachis oil BP. The degree of erythema at the injection sites was assessed approximately 24, 48, 72 hours and 7 days after injection according to the scale shown in Appendix 7. The degree of oedema was not evaluated. Any evidence of systemic toxicity was also recorded. The highest concentration that caused only mild to moderate skin irritation, and which was well tolerated systemically, was selected for the intradermal induction stage of the main study. The results are given in Appendix 1.

3.2.1.2 Selection of Concentration for Topical Induction

Two guinea pigs (intradermally injected with Freund's Complete Adjuvant fourteen days earlier) were treated with the undiluted test material and three preparations of the test material (75%, 50% and 25% v/v in arachis oil BP). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 48 hours. Due to the severity of dermal reactions, the degree of erythema and oedema was evaluated immediately after dressing removal and these two animals were humanely killed. Two additional animals (intradermally injected with Freund's Complete Adjuvant seventeen days earlier) were similarly treated with four lower concentrations of the test material (25%, 10%, 5% and 2% v/v in arachis oil BP). The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study. The results are given in Appendix 2.

3.2.1.3 Selection of Concentration for Topical Challenge

Four preparations of the test material (25%, 10%, 5% and 2% v/v in arachis oil BP) were applied to the clipped flanks of two guinea pigs under occlusive dressings for an exposure period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest non-irritant concentration of the test material, at the 24 and 48-hour observations, and one lower concentration were selected for the topical challenge stage of the main study. The results are given in Appendix 3.

3.2.2 Main Study

A group of thirty guinea pigs was used for the main study, twenty test and ten control. The bodyweight of each animal was recorded at the start and end of the study and are presented in Appendix 6.

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers. A row of three injections (0.1 ml each) was made on each side of the mid-line into a 20 mm x 40 mm area. The injections were:

a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1

b) a 1% v/v formulation of the test material in arachis oil BP

c) a 1% v/v formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water.

Approximately 24 and 48 hours after intradermal injection the degree of erythema at the test material injection sites (ie. injection site b) was evaluated according to the scale shown in Appendix 7.

On Day 7 the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the test material formulation. A filter paper patch (WHATMAN No.4: approximate size 40 mm x 20 mm), saturated with the test material formulation (10% v/v in arachis oil BP) was applied to the prepared skin and held in place with a strip of surgical adhesive tape covered with an overlapping length of aluminium foil. The patch and foil were further secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours.

The degree of erythema and oedema was quantified one and twenty-four hours following removal of the patches using the scale shown in Appendix 7. The individual reactions are given in Appendix 5.

Any other reactions were also recorded.

Induction of the Control Animals: The intradermal induction was performed using an identical procedure to that used for the test animals except that the test material was omitted from the intradermal injections. Injection b) was therefore the vehicle alone, injection c) was a 50% formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

3.2.2.2 Challenge

Shortly before treatment on Day 21, an area of approximately 50 mm x 70 mm on both flanks of each animal, was clipped free of hair with veterinary clippers.

A square filter paper patch (WHATMAN No.4: approximate size 20 mm x 20 mm), saturated with the test material formulation at the maximum non-irritant concentration (2% v/v in arachis oil BP) was applied to the shorn right flank of each animal and was held in place with a strip of surgical adhesive tape. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 1% v/v in arachis oil BP was similarly applied to a skin site on the left shorn flank. The patches were occluded with an overlapping length of aluminium foil and secured with a strip of elastic adhesive bandage wound in a double layer around the torso of each animal.

After 24 hours, the dressing was carefully removed and discarded. The challenge sites were swabbed with cotton wool soaked in diethyl ether to remove residual material. The position of the treatment sites was identified by using a black indelible marker-pen.

Prior to the 24-hour observation the flanks were clipped using veterinary clippers to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 7.

Any other reactions were also recorded.

3.2.2.3 Rechallenge

Fourteen days after the original challenge and at the request of the Sponsor, the test group animals were re-challenged on previously untreated areas of skin using the test material at concentrations of 2% and 1% v/v in arachis oil BP. An additional control group of ten animals was similarly treated. These control animals had not previously been exposed to the test material but had received intradermal injections of Freund's Complete Adjuvant. Approximately 24 and 48 hours after rechallenge dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix 7.

3.3 Interpretation of Results

Skin reactions noted at the challenge sites of the test group animals will be attributed to skin sensitisation, providing that reactions of equal severity are not seen at the corresponding challenge sites of the control group animals.

If skin reactions are seen at the challenge sites of the control group animals, these will be due to skin irritation, and therefore only skin reactions of greater severity in the test group animals will be attributed to skin sensitisation.

Barely perceptible erythema (grade ±) is often a non-specific response to the dosing procedure and is not considered to be a significant or conclusive indication of delayed contact hypersensitivity. Furthermore, transient challenge reactions (those which do not persist for at least 48 hours) will not be attributed to contact sensitisation.

The sensitisation potential of the test material will be classified as follows:

Percentage of sensitised animals	Classification
0	non-sensitiser
>0 – 8	weak sensitiser
>8 – 28	mild sensitiser
>28 – 64	moderate sensitiser
>64 – 80	strong sensitiser
>80 – 100	extreme sensitiser

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Skin Reactions Observed After Intradermal Induction

Individual skin reactions at the intradermal induction sites of the test and control group animals are given in Appendix 4.

Discrete or patchy to moderate and confluent erythema was noted at the intradermal induction sites of test and control group animals.

5.2 Skin Reactions Observed After Topical Induction

Individual skin reactions at the topical induction sites of the test and control group animals are given in Appendix 5.

Discrete or patchy to moderate and confluent erythema and very slight to slight oedema were noted at the topical induction sites of test group animals. A hardened dark brown/black coloured scab was noted at the topical induction sites of two test group animals. Dried blood was noted at the topical induction sites of three test group animals at the 24-hour observation. Skin reactions prevented evaluation of erythema and oedema at the topical induction site of one test group animal at the 1 and 24-hour observations. Residual test material was noted at the topical induction sites of seventeen test group animals at the 1-hour observation and at the topical induction sites of fourteen test group animals at the 24-hour observation.

Discrete or patchy erythema and very slight oedema were noted at the topical induction sites of control group animals. A sticky residue was noted at the topical induction sites of five control group animals at the 1 and/or 24-hour observations.

Bleeding from intradermal injection sites was noted in eighteen test group animals and six control group animals.

5.3 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the challenge sites of the test and control group animals are given in Table 1.

One test group animal was found dead on Day 17. The cause of death was not determined and was considered not to affect the purpose or integrity of the study.

2% v/v in Arachis Oil BP

Positive skin responses (discrete or patchy to moderate and confluent erythema, one with and three without very slight oedema) were noted at the topical challenge sites of four test group animals at the 24 and 48-hour observations. Desquamation was noted at the topical challenge sites of five test group animals at the 48-hour observation.

No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hour observations.

1% v/v in Arachis Oil BP

A positive skin response (moderate and confluent erythema and very slight oedema) was noted at the topical challenge site of one test group animal at the 24 and 48-hour observations. Desquamation was noted at the topical challenge site of one test group animal at the 48-hour observation.

No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hour observations.

5.4 Skin Reactions Observed After Topical Rechallenge

Individual skin reactions at the rechallenge sites of the test and control group animals are given in Table 2.

2% v/v in Arachis Oil BP

Positive skin responses were noted at the topical challenge sites of eight test group animals. Discrete or patchy to moderate and confluent erythema was noted at the topical challenge sites of nine test group animals at the 24-hour observation and in four test group animals at the 48-hour observation. Discrete or patchy erythema noted in one test group animal at the 24-hour observation was not apparent at the 48-hour observation. Therefore, this was considered not to be attributed to contact sensitisation. Other skin reactions noted were desquamation or severe desquamation and crust formation. Skin reactions prevented evaluation of oedema and/or erythema at the topical challenge sites of four test group animals at the 48-hour observation.

No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hour observations.

1% v/v in Arachis Oil BP

Positive skin responses (discrete or patchy to moderate and confluent erythema) were noted at the topical challenge sites of two test group animals at the 24 and 48-hour observations. Transient challenge reactions (discrete or patchy erythema) were noted at the topical challenge sites of two test group animals at the 24-hour observation. These reactions were not apparent at the 48-hour observation and were therefore not attributed to contact sensitisation. Desquamation was noted at the topical challenge sites six test group animals at the 48-hour observation.

No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hour observations.

6. CONCLUSION

Under the conditions of the test, the test material produced a positive response in 21% (4/19) animals following topical challenge at a concentration of 2% v/v in arachis oil BP. At topical rechallenge a positive response was noted in 42% (8/19) animals.

Table 1 **Individual Skin Reactions at Challenge**

CHALLENGE CONCENTRATIONS: **2% and 1% v/v**

VEHICLE: **Arachis Oil BP**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		24 Hours						48 Hours					
		1%			2%			1%			2%		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
TEST	1	2	1	-	2	1	-	2	1	D	2	1	D
	2	0	0	-	1	0	-	0	0	-	1	0	D
	3	0	0	-	0	0	-	0	0	-	0	0	-
	4	0	0	-	0	0	-	0	0	-	0	0	D
	5	0	0	-	1	0	-	0	0	-	1	0	-
	6	0	0	-	0	0	-	0	0	-	0	0	-
	7	0	0	-	0	0	-	0	0	-	0	0	-
	8	0	0	-	1	0	-	0	0	-	1	0	-
	9	0	0	-	0	0	-	0	0	-	0	0	D
	10	0	0	-	0	0	-	0	0	-	0	0	-
	11	0	0	-	0	0	-	0	0	-	0	0	-
	12	0	0	-	0	0	-	0	0	-	0	0	-
	13	0	0	-	0	0	-	0	0	-	0	0	-
	14	0	0	-	0	0	-	0	0	-	0	0	-
	15	0	0	-	0	0	-	0	0	-	0	0	D
	16	0	0	-	0	0	-	0	0	-	0	0	-
	17	0	0	-	0	0	-	0	0	-	0	0	-
	18	0	0	-	0	0	-	0	0	-	0	0	-
	19	0	0	-	0	0	-	0	0	-	0	0	-
	20	*	*	*	*	*	*	*	*	*	*	*	*

Er = Erythema Oe = Oedema - = No other reactions noted * = No data, animal found dead Day 17 D = Desquamation

Table 1 (continued) Individual Skin Reactions at Challenge

CHALLENGE CONCENTRATIONS: **2% and 1% v/v**

VEHICLE: **Arachis Oil BP**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		24 Hours						48 Hours					
		1%			2%			1%			2%		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
CONTROL	21	0	0	-	0	0	-	0	0	-	0	0	-
	22	0	0	-	0	0	-	0	0	-	0	0	-
	23	0	0	-	0	0	-	0	0	-	0	0	-
	24	0	0	-	0	0	-	0	0	-	0	0	-
	25	0	0	-	0	0	-	0	0	-	0	0	-
	26	0	0	-	0	0	-	0	0	-	0	0	-
	27	0	0	-	0	0	-	0	0	-	0	0	-
	28	0	0	-	0	0	-	0	0	-	0	0	-
	29	0	0	-	0	0	-	0	0	-	0	0	-
	30	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

Table 2 **Individual Skin Reactions at Rechallenge**

RECHALLENGE CONCENTRATIONS: **2% and 1% v/v**

VEHICLE: **Arachis Oil BP**

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		24 Hours						48 Hours					
		1%			2%			1%			2%		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
TEST	1	2	0	-	2	1	D	2	0	D	?e	?od	Cf
	2	1	0	-	1	0	-	0	0	-	1	0	D
	3	0	0	-	0	0	-	0	0	-	0	0	-
	4	0	0	-	2	1	-	0	0	D	?e	1	D+
	5	0	0	-	1	0	D	0	0	D	1	0	D
	6	0	0	-	0	0	-	0	0	-	0	0	-
	7	0	0	-	2	0	D	0	0	-	?e	0	D+
	8	1	0	-	2	1	-	0	0	D	?e	0	D+
	9	0	0	-	2	0	-	0	0	D	1	0	D
	10	0	0	-	0	0	-	0	0	-	0	0	-
	11	0	0	-	0	0	-	0	0	-	0	0	-
	12	0	0	-	0	0	-	0	0	-	0	0	-
	13	0	0	-	0	0	-	0	0	-	0	0	-
	14	0	0	-	0	0	-	0	0	-	0	0	-
	15	2	0	-	2	1	D	2	0	D	2	1	D
	16	0	0	-	0	0	-	0	0	-	0	0	-
	17	0	0	-	1	0	-	0	0	-	0	0	-
	18	0	0	-	0	0	-	0	0	-	0	0	-
	19	0	0	-	0	0	-	0	0	-	0	0	-
	20	*	*	*	*	*	*	*	*	*	*	*	*

Er = Erythema Oe = Oedema - = No other reactions noted D = Desquamation

* No data, animal found dead Day 17 Cf = Crust formation D+ = Severe desquamation

?e = Adverse reactions prevented evaluation of erythema ?od = Adverse reactions prevented evaluation of oedema

Table 2 (continued) Individual Skin Reactions at Rechallenge

RECHALLENGE CONCENTRATIONS: 2% and 1% v/v

VEHICLE: Arachis Oil BP

Group	Animal Number	Skin Reactions (Hours after Removal of Dressings)											
		24 Hours						48 Hours					
		1%			2%			1%			2%		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
CONTROL	21R	0	0	-	0	0	-	0	0	-	0	0	-
	22R	0	0	-	0	0	-	0	0	-	0	0	-
	23R	0	0	-	0	0	-	0	0	-	0	0	-
	24R	0	0	-	0	0	-	0	0	-	0	0	-
	25R	0	0	-	0	0	-	0	0	-	0	0	-
	26R	0	0	-	0	0	-	0	0	-	0	0	-
	27R	0	0	-	0	0	-	0	0	-	0	0	-
	28R	0	0	-	0	0	-	0	0	-	0	0	-
	29R	0	0	-	0	0	-	0	0	-	0	0	-
	30R	0	0	-	0	0	-	0	0	-	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted

Appendix 1 Intradermal Sighting Test – Summary of Results

VEHICLE: Arachis Oil BP

Animal Identification	Time of Observation	Concentration of Test Material (% v/v)	Grade of Erythema at Injection Sites	Evidence of Systemic Toxicity
A	24 Hours	1	2	None
	48 Hours		2	None
	72 Hours		1	None
	7 Days		0	None
B	24 Hours	5	N	None
	48 Hours		NE	None
	72 Hours		E	None
	7 Days		E	None

The concentration of the test material selected for the intradermal induction stage of the main study was 1% v/v in arachis oil BP

N = Pale green coloured dermal necrosis E = Eschar

Appendix 2 Topical Sighting Test for Induction Application (48-Hour Exposure) – Individual Skin Reactions

VEHICLE: **Arachis Oil BP**

Animal Identification	Concentration of Test Material (% v/v)	Immediately after Removal of Patches		
		Er	Oe	Other
C	100	N	3	-
	75	N	3	-
	50	N	2	-
	25	2	1	-
D	100	N	3	-
	75	N	3	-
	50	N	3	-
	25	N	3	-

Er = Erythema Oe = Oedema - = No other reactions noted N= Dark green coloured dermal necrosis

Appendix 2 (continued) Topical Sighting Test for Induction Application (48-Hour Exposure) – Individual Skin Reactions

VEHICLE: **Arachis Oil BP**

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)								
		1			24			48		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
G	25	2	1	Bl	2	1	Bl	2	1	D
	10	2	0	-	1	0	-	0	0	-
	5	1	0	-	1	0	-	0	0	-
	2	1	0	-	0	0	-	0	0	-
H	25	2	1	Bl	2	0	Bl	2	0	Br
	10	2	0	-	1	0	-	0	0	-
	5	1	0	-	0	0	-	0	0	-
	2	1	0	-	0	0	-	0	0	-

The concentration of the test material selected for the main study topical induction was 10% v/v in arachis oil BP

Er = Erythema
Bl = Blanching of the skin
Oe = Oedema
D = Desquamation
- = No other reactions noted
Br = Light brown discolouration of the epidermis

Appendix 3 Topical Sighting Test for Challenge Application (24-Hour Exposure) – Individual Skin Reactions

VEHICLE: **Arachis Oil BP**

Animal Identification	Concentration of Test Material (% v/v)	Skin Reactions (Hours After Removal of Patches)								
		1			24			48		
		Er	Oe	Other	Er	Oe	Other	Er	Oe	Other
E	25	2	0	-	1	0	-	1	0	Le
	10	1	0	-	1	0	-	0	0	-
	5	1	0	-	1	0	-	0	0	-
	2	1	0	-	0	0	-	0	0	-
F	25	2	0	-	1	0	-	1	0	-
	10	1	0	-	0	0	-	0	0	-
	5	1	0	-	0	0	-	0	0	-
	2	1	0	-	0	0	-	0	0	-

The concentrations of the test material selected for the main study topical challenge were 2% and 1% v/v in arachis oil BP

Er = Erythema Oe = Oedema - = No other reactions noted Le = Loss of skin elasticity

Appendix 4 Intradermal Induction – Individual Skin Reactions

Group	Animal Number	Grade of Erythema at Observation Site			
		24 Hours		48 Hours	
		Left Side	Right Side	Left Side	Right Side
TEST	1	2	2	2	2
	2	2	2	2	2
	3	2	2	2	2
	4	2	2	2	2
	5	2	2	2	2
	6	2	2	2	2
	7	2	2	2	1
	8	2	2	2	2
	9	2	2	2	2
	10	2	2	2	2
	11	2	2	2	2
	12	2	2	2	2
	13	2	2	2	2
	14	2	2	2	2
	15	2	2	2	2
	16	2	2	2	2
	17	2	2	2	2
	18	2	2	2	2
	19	2	2	2	2
	20	2	2	2	2

Appendix 4 (continued) Intradermal Induction – Individual Skin Reactions

Group	Animal Number	Grade of Erythema at Observation Site			
		24 Hours		48 Hours	
		Left Side	Right Side	Left Side	Right Side
CONTROL	21	2	2	2	2
	22	2	2	1	1
	23	2	2	1	1
	24	2	2	1	1
	25	2	2	1	1
	26	2	2	1	1
	27	2	2	1	1
	28	2	2	1	1
	29	2	2	2	1
	30	1	1	1	1

Appendix 5 Topical Induction – Individual Skin Reactions

Group	Animal Number	Skin Reactions (Hours After Removal of Dressing)					
		1 Hour			24 Hours		
		Er	Oe	Other	Er	Oe	Other
TEST	1	2	2	BsRt	2	2	StRt
	2	?e	?od	Bs	?e	?od	St
	3	2	1	BsRt	1	1	Rt
	4	2	1	BsRt	1	1	Rt
	5	2	1	BsRt	1	0	-
	6	2	1	BsRt	1	0	RtBd
	7	2	0	BsRt	1	0	Rt
	8	2	1	BsRt	1	0	Rt
	9	2	0	BsRt	1	0	RtBd
	10	2	1	BsRt	1	0	Rt
	11	1	0	Bs	1	0	-
	12	2	2	BsRt	1	0	Bd
	13	2	0	Rt	1	0	Rt
	14	2	0	Rt	1	0	Rt
	15	2	1	BsRt	1	0	Rt
	16	2	1	BsRt	1	0	Rt
	17	2	2	BsRt	1	0	-
	18	2	0	BsRt	1	0	Rt
	19	2	0	Bs	1	0	-
	20	2	1	BsRt	1	0	Rt

Er = Erythema Oe = Oedema - = No other reactions noted Bs = Bleeding from intradermal injection sites
Rt = Residual test material Bd = Dried blood St = A hardened dark brown/black coloured scab
?e = Adverse reactions prevented evaluation of erythema ?od = Adverse reactions prevented evaluation of oedema

Appendix 5 (continued) Topical Induction – Individual Skin Reactions

Group	Animal Number	Skin Reactions (Hours After Removal of Dressing)					
		1 Hour			24 Hours		
		Er	Oe	Other	Er	Oe	Other
CONTROL	21	1	0	-	1	0	-
	22	1	0	-	0	0	-
	23	1	0	-	1	0	-
	24	1	0	-	1	0	-
	25	1	0	Bs	0	0	-
	26	1	0	Bs	1	0	Rt+
	27	1	1	BsRt+	1	0	Rt+
	28	1	1	BsRt+	0	0	Rt+
	29	1	1	BsRt+	1	0	Rt+
	30	1	1	BsRt+	0	0	-

Er = Erythema Oe = Oedema - = No other reactions noted
Bs = Bleeding from intradermal injection sites Rt+ = Sticky residue

Appendix 6 Individual Bodyweights and Bodyweight Change

Group	Animal Number	Bodyweight (g)				Bodyweight (g) Change	
		Day 0	Day 24	Day 35	Day 38	Day 0 - 24	Day 35 - 38
TEST	1	411	527	585	585	116	0
	2	412	556	642	637	144	-5
	3	422	569	673	652	147	-21
	4	374	545	625	613	171	-12
	5	420	561	646	639	141	-7
	6	398	546	633	623	148	-10
	7	422	490	616	598	68	-18
	8	386	556	637	638	170	1
	9	417	539	609	599	122	-10
	10	384	547	647	643	163	-4
	11	412	591	663	651	179	-12
	12	377	526	610	583	149	-27
	13	439	674	803	772	235	-31
	14	449	586	682	647	137	-35
	15	450	624	738	714	174	-24
	16	490	664	792	775	174	-17
	17	401	544	620	611	143	-9
	18	440	605	707	680	165	-27
	19	441	621	712	701	180	-11
	20	440	*	*	*	*	*

Bodyweight changes of the guinea pigs in the test group animals were comparable to those noted in the control group animals during the study.

* = No data, animal found dead Day 17

Appendix 6 (continued) Individual Bodyweights and Bodyweight Change

Group	Animal Number	Bodyweight (g)				Bodyweight (g) Change	
		Day 0	Day 24	Day 35	Day 38	Day 0 - 24	Day 35 - 38
CONTROL	21	435	618	-	-	183	-
	22	417	591	-	-	174	-
	23	392	530	-	-	138	-
	24	378	539	-	-	161	-
	25	437	633	-	-	196	-
	26	378	528	-	-	150	-
	27	418	545	-	-	127	-
	28	429	618	-	-	189	-
	29	383	512	-	-	129	-
	30	397	566	-	-	169	-
	21R	-	-	813	794	-	-19
	22R	-	-	821	822	-	1
	23R	-	-	706	704	-	-2
	24R	-	-	687	747	-	60
	25R	-	-	645	680	-	35
	26R	-	-	651	641	-	-10
	27R	-	-	633	646	-	13
	28R	-	-	666	686	-	20
	29R	-	-	664	653	-	-11
	30R	-	-	631	618	-	-13

- = Not applicable

Appendix 7 Scales For Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA #	VALUE
No erythema	0
Barely perceptible erythema	±
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

EVALUATION OF OEDEMA †	VALUE
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre extending beyond the area of exposure)	4

From: Modified OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

† From: Draize, J H (1977) "Dermal and Eye Toxicity Tests" In: Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington DC, p31.

Appendix 8 Summary of Positive Control Data for the Magnusson and Kligman Maximisation Study

Project Number	Date Start	Date End	Number of Animals and Sex*		Positive Control Material	Concentration			Incidence of Sensitisation
						Induction		Challenge	
			Test	Control		Intradermal	Topical		
039/333	22/12/98	05/02/99	10 Female	5 Female	2-Mercaptobenzothiazole	10% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	90% (9/10)
039/370	14/06/99	17/07/99	10 Male	5 Male	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (10/10)
039/422	12/01/00	05/02/00	10 Female	5 Female	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (10/10)
039/444	29/06/00	22/07/00	10 Male	5 Male	2-Mercaptobenzothiazole	5% in arachis oil BP	50% in acetone:PEG 400 (70:30)	50% and 25% in acetone:PEG 400 (70:30)	100% (9/9)
039/446	28/06/00	06/08/00	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	50% (5/10)
039/458	25/01/01	25/02/01	10 Male	5 Male	α-Hexylcinnamaldehyde	5% in arachis oil BP	100%	100% and 75% in arachis oil BP	40% (4/10)

* All animals supplied by David Hall Ltd, Burton-on-Trent, Staffordshire, UK

Appendix 9 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Ltd
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE

Analytical Chemistry
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Tests
Toxicology

DATE OF INSPECTION
28 February 2000

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

Appendix 10 Copy of Protocol

SafePharm Laboratories

PROTOCOL

TEST MATERIAL	:	
STUDY TYPE	:	Skin Sensitisation (Magnusson & Kligman Maximisation) Study in the Guinea Pig
TEST METHOD	:	575.09
PROJECT NUMBER	:	1014/135
PROPOSED START DATE	:	Mid August 2001
PROPOSED COMPLETION DATE	:	Mid September 2001
TARGET (DRAFT) REPORT DATE	:	End of September 2001
SPONSOR	:	

SPECIAL CONDITIONS : If any skin reactions are observed in the test animals, the Sponsor will be contacted for a possible rechallenge prior to sacrifice of the animals.

This study is compliant with the following toxicology guidelines:

- OECD Test Guideline 406, 1992
- Method B6 Skin Sensitisation of Commission Directive 96/54/EC
- Japanese MHW, 1992
- US EPA OPPTS 870.2600, 1998

APPROVED FOR SPONSOR BY: _____ DATE: 7/30/01

AUTHORISED BY: _____ DATE: 14 AUG 2001

P Brunt HNC
STUDY DIRECTOR

This protocol is issued without signature by the Study Director to enable changes to be made if necessary prior to authorisation. Sponsors should sign and return the document to indicate approval and GLP authorisation will be confirmed by the Study Director's signature prior to the start of the study.

SKIN SENSITISATION (MAGNUSSON & KLIGMAN MAXIMISATION) STUDY IN THE GUINEA PIG

1. INTRODUCTION AND OBJECTIVES

To assess the skin sensitisation potential of the test material in the guinea pig. The maximisation study is an 'adjuvant' type test in which sensitisation is potentiated by the intradermal injection of Freund's Complete Adjuvant. The results of the study are believed to be of value in predicting the likely skin sensitisation potential of the test material to man.

The work will be performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM (98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

2. TEST FACILITY

Safepharm Laboratories Ltd
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

3. ANIMALS

Specification

Male or female albino Dunkin-Hartley strain guinea pigs obtained from David Hall Limited, Burton-on-Trent, Staffordshire, UK or other suitable accredited supplier. Females will be nulliparous and non-

pregnant. At the start of the main study, the animals will weigh 300 to 450g and are expected to be eight to twelve weeks old.

Number

Twenty test and ten control animals will be used for the main study.

Justification

Preferred species of choice as historically used for skin sensitisation studies and the strain used has been shown to produce satisfactory sensitisation response using known positive sensitisers at these laboratories.

4. ANIMAL HUSBANDRY

Environment

Target temperature:	17 - 23°C
Target humidity:	30 - 70%
Lighting:	twelve hours of continuous artificial light in each twenty-four hour period
Ventilation:	at least fifteen air changes per hour

Housing

Housed singly or in pairs in suspended solid-floor polypropylene cages fitted with stainless steel mesh lids and furnished with softwood woodflakes. Results of routine analysis of the woodflakes are made available to Safepharm Laboratories Ltd.

Diet and Water

Guinea Pig Diet FD1, Special Diets Services Limited, Witham, Essex, UK (or suitable alternative) and tap water *ad libitum*.

The diet and water are routinely analysed and are considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

5. ANIMAL WELFARE

Environmental Enrichment

Animals will be provided with environmental enrichment items: irradiated hay (Harlan UK Ltd. Bicester, Oxford) or suitable alternatives.

Study Conduct

The study was designed and will be conducted to cause the minimum suffering or distress to the animals consistent with the scientific objectives and in accordance with the Safepharm policy on animal welfare. This protocol is subject to review and the conduct of the study may be retrospectively reviewed, as part of the Safepharm Ethical Review Process.

6. PRE-TEST PROCEDURES

Acclimatisation Period

At least five days.

Identification

Each animal, selected at random, will be uniquely identified by indelible marker on small area of clipped rump. A cage card will be prepared showing details of test material, Safepharm serial number, project number, sex, number of animals, study dates and initials of the Study Director.

7. TEST MATERIAL AND EXPERIMENTAL PREPARATION

Supply

Supplied by Sponsor with details of hazardous properties if known. Data relating to the identification, purity and stability of the test material will be the responsibility of the Sponsor.

Storage

Room temperature in the dark unless otherwise specified by Sponsor.

Preparation

For intradermal injection, the test material will be dissolved, emulsified or suspended in a suitable vehicle. If possible, an aqueous formulation will be prepared. However, if water is found to be an unsatisfactory vehicle, preparation using other suitable vehicles will be attempted. For the intradermal induction phase of the main study, incorporation of the test material in a 1:1 mixture (v/v) of FCA (Freund's Complete Adjuvant) and distilled water will also be necessary.

For topical applications, liquid test materials may be administered undiluted, if appropriate. When dilution of the test material is necessary, this will firstly be attempted using distilled water. If distilled water is shown to be an unsatisfactory vehicle then dilution using other suitable vehicles will be attempted.

Solids may be finely pulverised before preparation of formulations for intradermal and topical administration. The vehicles used for dilution will be documented in the study file, together with a description of the method of preparation of the test material.

Analysis

All formulations will be used within two hours of preparation and will be assumed to be stable for this period unless specified otherwise by the Sponsor. The concentration and homogeneity of the formulations will not be determined by analysis.

Absorption

The absorption of the test material will not be determined.

8. SELECTION OF CONCENTRATIONS FOR MAIN STUDY (PRELIMINARY "SIGHTING" TESTS)

Intradermal Induction

Initially, individual guinea pigs will be intradermally injected with either a 1% or 5% preparation of the test material (four 0.1 ml injections). If necessary, lower or higher concentrations (up to a maximum concentration of 25%) may then be investigated using additional guinea pigs. Animals will be examined approximately 24, 48 and 72 hours after injection and then seven days after injection. The degree of erythema at the injection sites will be evaluated according to the 0 to 3 scale shown in the Appendix. The degree of oedema will not be evaluated. The highest concentration shown to be well tolerated

systemically, and causing only mild to moderate skin irritation, will be selected for the intradermal induction phase of the main study.

Topical Induction

Four concentrations of test material will be occlusively applied to the clipped flanks of two guinea pigs. These guinea pigs will have been intradermally injected with FCA at least seven days earlier.

Liquids will be applied at concentrations of 100%, 75%, 50% and 25%. Solids will be applied at the maximum attainable concentration suitable for topical application (up to a maximum concentration of 75%) plus three lower concentrations. Patches and dose volumes will be the same as for the topical induction stage of the main study. The exposure period will be 48 hours. The degree of erythema and oedema will be evaluated 1, 24 and 48 hours after patch removal using the schemes shown in the Appendix.

If the test material is suspected to be very irritant, or toxic by the dermal route, then lower concentrations may be investigated and/or fewer patches will be applied to each animal.

The highest concentration producing only mild to moderate dermal irritation, will be selected for the topical induction stage of the main study.

Topical Challenge

A maximum of four concentrations of test material will be occlusively applied to the clipped flanks of two guinea pigs. These guinea pigs will not form part of the main study, but will have been treated identically to the Control Group animals of the main study, up to Day 14. Patches and dosages will be the same as for the topical challenge stage of the main study. The exposure period will be 24 hours. The degree of erythema and oedema will be evaluated 1, 24 and 48 hours after patch removal. The highest concentration producing no evidence of skin irritation at the 24 and 48 hour observations (the maximum non-irritant concentration), and one lower concentration will be selected for the topical challenge stage of the main study.

9. MAIN STUDY

The procedure may be considered in two parts, Induction and Challenge.

Induction of Test Group Animals

Treatment Site Preparation

Shortly before treatment on Day 0, an area approximately 40 mm x 60 mm on the shoulder region will be clipped free of hair with veterinary clippers. Care will be taken to avoid abrasion of the skin.

Intradermal Injections Day 0

Three pairs of intradermal injections will be given in the shoulder region, within an area of skin measuring approximately 20 mm x 40 mm. Injections of 0.1 ml volume will be made so that one injection of each pair lies on each side of the midline. Injections will therefore be:

Injection 1: A 1:1 mixture (v/v) of FCA/distilled water.

Injection 2: The test material in appropriate vehicle at the selected concentration.

Injection 3: The test material at the selected concentration formulated in a 1:1 mixture (v/v) of FCA/distilled water.

In injection 3, water soluble test materials will be dissolved in the aqueous phase prior to mixing with FCA. Liposoluble or insoluble test materials will be incorporated in FCA prior to combining with the aqueous phase. The final concentration of test material will be equal to that used in injection 2.

Injections 1 and 2 will be made close to each other and nearest the head. Injection 3 will be given towards the caudal part of the test area.

Evaluation of Irritant Effects (Day 1 and 2)

Approximately 24 and 48 hours after intradermal injection, the degree of erythema at the test material injection sites (injection 2) will be evaluated according to the 0-3 scale shown in the Appendix. The degree of oedema will not be evaluated.

Topical Induction (Day 7)

The induction site will again be clipped free of hair. A filter paper patch (WHATMAN No.4; approximate size 20 mm x 40 mm) will be loaded with the undiluted test material or the test material formulated in a suitable vehicle. Liquid test materials will be applied to saturation. Pastes or slurries will be applied to the

patch to form a thick, even layer. The loaded patch will be applied to the clipped induction site and held in place with a strip of impermeable surgical adhesive tape. The patch will be covered with an overlapping length of aluminium foil and secured with a strip of elastic adhesive bandage wound in a double layer around the shoulder region.

Removal of Induction Dressings (Day 9)

Approximately 48 hours after application, the occlusive dressings will be removed from each test group animal.

Evaluation of skin Reactions

Approximately 1 and 24 hours after dressing removal the degree of erythema and oedema at the induction site will be evaluated.

Induction of Control Group Animals

Intradermal Injections (Day 0)

Injections will be administered using the procedures described for the test group animals except that the pairs of injections will be:

Injection 1: A 1:1 mixture (v/v) of FCA/distilled water.

Injection 2: Vehicle alone.

Injection 3: A 50% formulation of vehicle in a 1:1 mixture (v/v) of FCA/distilled water.

Topical Induction (Day 7)

Control animals will be treated identically to the test group animals except that they will not be exposed to the test material. If undiluted liquids are applied to the test group animals, the filter paper applied to the control animals will remain untreated. If a formulation of test material is applied to the test group animals, the vehicle will be loaded onto the filter paper before application to the control animals.

Removal of Induction Dressings (Day 9)

Approximately 48 hours after application, the occlusive dressings will be removed from each control group animal.

Evaluation of Skin Reactions

Skin reactions will be evaluated approximately 1 and 24 hours after dressing removal, as described for the test group animals.

Topical Challenge of Test and Control Group Animals

Treatment Site Preparation

Shortly before treatment on Day 21, an area approximately 50 mm x 70 mm on both flanks of each animal will be clipped free of hair. Care will be taken to avoid abrasion of the skin.

Application (Day 21)

A filter paper patch (WHATMAN NO. 4; approximate size 20 mm x 20 mm) will be loaded with the test material at the maximum non-irritant concentration, and will be applied to the clipped right flank. The patch will be held in place with a strip of impermeable surgical adhesive tape. A second filter paper patch (approximate size 20 mm x 20 mm) will be loaded with the test material prepared at a lower concentration, and will be applied to the clipped left flank. This patch will also be held in place with a strip of surgical adhesive tape. If considered necessary, the vehicle alone may be also be applied to a separate skin site on one of the clipped flanks.

All patches will be covered with an overlapping length of aluminium foil and secured by a strip of elastic adhesive bandage.

Removal of Challenge Dressings (Day 22)

Approximately 24 hours after application, all occlusive dressings will be carefully removed. The challenge sites will be gently swabbed using cotton wool soaked in distilled water or other suitable solvent, to remove excess test material. Care will be taken not to alter the existing skin response. The position of the challenge sites will be identified using a black indelible marker-pen.

Evaluation of Challenge Reactions

Approximately 21 hours after removal of the patches, the challenge sites will be clipped free of hair, and if necessary, the skin swabbed once again. Clipping may also be performed on the morning prior to the 48 hour observation.

Approximately 24 and 48 hours after patch removal, the degree of erythema and oedema will be evaluated according to the schemes shown in the Appendix. Any other skin reactions will also be recorded. If considered necessary, for example, if delayed responses are noted or if reactions appear to be increasing in severity, additional daily observations may be made in order to ensure that the maximum sensitisation response is identified.

10. CLINICAL OBSERVATIONS

Bodyweights

Recorded at the start of the study and at termination.

Health effects

Signs of systemic toxicity or other adverse health effects will be documented in the study file.

Histopathology

If, at challenge, assessment of the sensitisation response is precluded by staining of the skin by the test material, histopathological examination of the skin sites may be necessary to confirm the presence or absence of sensitisation responses. Histopathology will only be performed after consultation with, and at extra cost to the study sponsor.

11. INTERPRETATION OF RESULTS

Skin reactions noted at the challenge sites of the test group animals will be attributed to skin sensitisation, providing that reactions of equal severity are not seen at the corresponding challenge sites of the control group animals.

If skin reactions are seen at the challenge sites of the control group animals, these will be due to skin irritation, and therefore only skin reactions of greater severity in the test group animals will be attributed to skin sensitisation.

Barely perceptible erythema (grade ±) is often a non-specific response to the dosing procedure and is considered not to be a significant or conclusive indication of delayed contact hypersensitivity.

Furthermore, transient challenge reactions (those which do not persist for at least 48 hours) will also not be attributed to contact sensitisation.

The sensitisation potential of the test material will be classified as follows:

Percentage of sensitised animals	**Classification**
0	non-sensitiser
> 0 - 8	weak sensitiser
> 8 - 28	mild sensitiser
> 28 - 64	moderate sensitiser
> 64 - 80	strong sensitiser
> 80 - 100	extreme sensitiser

12. RE-CHALLENGE

If it is necessary to clarify the results of the topical challenge, a second challenge may be performed using the same test group animals. If appropriate, a fresh group of control animals (previously intradermally injected with FCA but not exposed to the test material) can be treated at re-challenge. The re-challenge will be performed approximately one week after the first challenge and applications will be made to previously untreated areas of skin. Re-challenge will only be carried out after consultation with, and at extra cost, to the Sponsor.

13. POSITIVE CONTROL DATA

The sensitivity and reliability of the test system will be checked at least every six months using substances which are known to have mild to moderate skin sensitisation properties. Preferred substances are α-hexyl cinnamicaldehyde (CAS No. 101-86-0), mercaptobenzothiazole (CAS No. 149-30-4) and benzocaine (CAS No. 94-09-7). A sensitisation response in at least 30% of animals tested is expected for these substances.

14. QUALITY ASSURANCE

This standard test method will be reviewed for GLP compliance and the final report will be audited by Safepharm Quality Assurance Unit. This type of study is subject to process-based QA inspection designed to encompass the major phases once per month.

15. PROTOCOL AMENDMENTS

Amendments to protocol will be made only by completion of an amendment to protocol form authorised by the Study Director.

16. FINAL REPORT

The final report will include, as a minimum, the following information:

Details of test material
Details of vehicles used
Species, strain, supplier, number, age and sex of animals
Environmental conditions and animal diet
Test conditions
Record of all individual observations in tabular form
Narrative description of the nature and degree of the effects observed
Discussion of results
Summary of the latest reliability check including information on the substance, concentration and vehicle used

17. ARCHIVE

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for a period of five years after which instructions will be sought as to further retention or disposal. Further retention or return of the data will be chargeable to the Sponsor.

18. REFERENCES

Magnusson B, and Kligman A M (1969) The identification of contact allergens by animal assay. The guinea pig maximisation test. *J of Investigative Dermatology* 52, 268 - 276.

Magnusson B, and Kligman A M (1970) Identification of contact allergens. In: *Allergic contact dermatitis in the guinea pig*. Springfield, Ill, p 102 - 124.

Magnusson B (1975) The relevance of results obtained with the guinea pig maximisation test. In: *Animal models in dermatology* (Maibach H ed), pp 76 - 83, Edinburgh, Churchill Livingstone.

Magnusson B, Fregert S and Wahlberg J (1979) Determination of skin sensitisation potential of chemicals. *Predictive testing in guinea pigs*. Arbete och Hälsa, 26(E).

Magnusson B (1980). Identification of contact sensitisers by animal assay. *Cont. Derm.*, 6, 46.

ECETOC Technical Report No.78 (1999) Skin Sensitisation Testing : Methodological Considerations.

Appendix 1 Scales For Evaluation of Skin Reactions

EVALUATION OF ERYTHEMA	VALUE
No erythema	0
Barely perceptible erythema	±
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Adapted from: OECD Test Guideline 406, 1992 and Method B6 Skin Sensitisation of Commission Directive 96/54/EC.

EVALUATION OF OEDEMA	VALUE
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre extending beyond the area of exposure)	4

From: Draize, J H (1977) "Dermal and Eye Toxicity Tests" In: Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington DC, p31.

SafePharm Laboratories

ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 1014/132

AUTHOR: P Brunt

STUDY SPONSOR:

TEST FACILITY:

Safepharm Laboratories Limited
P.O. Box No. 45
DERBY
DE1 2BT
U.K.

Telephone: (01332) 792896

Facsimile: (01332) 799018

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	23 December 1999	Standard Test Method Compliance Audit
	07 August 2001	Test Material Preparation
	23 August 2001	Animal Preparation
	14 August 2001	Dosing
	07 August 2001	Assessment of Response
	14 August 2001	Necropsy
§	10 September 2001	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 28 NOV 2001

For Safepharm Quality Assurance Unit*

---

*Authorised QA Signatures:

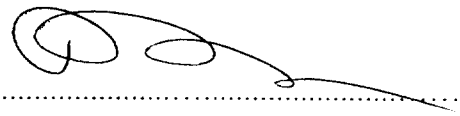
Head of Department: JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: JV Johnson BSc MRQA; G Wren ONC MRQA; R Hurst MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.



..... DATE: 23 NOV 2001

P Brunt HNC
Study Director

CONTENTS

ACUTE ORAL TOXICITY IN THE RAT – ACUTE TOXIC CLASS METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 22 March 1996)

- Commission Directive 96/54/EC Method B1 tris Acute Oral Toxicity (Oral – Acute Toxic Class Method)

Method. A group of three fasted females was treated with the test material at a dose level of 2000 mg/kg bodyweight. Based on the results from this dose level further groups of fasted animals were treated at a dose level of 200 mg/kg bodyweight. Dosing was performed sequentially.

For the 2000 mg/kg dose level the test material was administered orally undiluted and for the 200 mg/kg dose level the test material was administered orally as a solution in arachis oil BP. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. Female animals treated at a dose level of 2000 mg/kg were found dead thirty minutes or one hour after dosing. There were no deaths noted in animals treated at a dose level of 200 mg/kg.

Clinical Observations. Signs of systemic toxicity noted for the female treated at 2000 mg/kg during the study were exophthalmos, clonic convulsions, decreased respiratory rate, laboured and noisy respiration. Exophthalmos, lethargy and occasional body tremors were noted in animals treated at 200 mg/kg during the day of dosing. Hunched posture was also noted until two days after dosing.

Bodyweight. The surviving animals showed expected gains in bodyweight over the study period.

Necropsy. Abnormalities noted at necropsy of the females treated at a dose level of 2000 mg/kg that died during the study were haemorrhagic lungs, dark liver, dark kidneys and slight haemorrhage of the small intestine. No abnormalities were noted at necropsy of animals treated at a dose level of 200 mg/kg that were killed at the end of the study.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rat was estimated to be in the range of 300 - 500 mg/kg bodyweight.

The test material was classified as HARMFUL and the symbol "Xn" and risk phrase R 22 "HARMFUL IF SWALLOWED" are required according to EU labelling regulations Commission Directive 93/21/EEC.

ACUTE ORAL TOXICITY IN THE RAT
- ACUTE TOXIC CLASS METHOD

1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 22 March 1996)

- Commission Directive 96/54/EC Method B1 tris Acute Toxicity (Oral – Acute Toxic Class Method)

The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 09 August 2001 and 04 September 2001.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	
Chemical name	:	
CAS number	:	
% Solid	:	97.8% (2.1% Toluene, 0.1% water)
Description	:	yellow coloured liquid
Lot Number	:	03817
Date received	:	30 April 2001
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of the 2000 mg/kg dose level the test material was used as supplied. The specific gravity was determined and used to calculate the appropriate dose volume for the required dose level.

For the purpose of the 200 mg/kg dose level the test material was freshly prepared, as required, as a solution at the appropriate concentration in arachis oil BP.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Male and female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rats were supplied by Charles River (UK) Ltd, Margate, Kent, UK. On receipt the animals were randomly allocated to cages. The females were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals weighed at least 200g, and were approximately eight weeks of age.

The animals were housed in groups of three by sex in solid-floor polypropylene cages furnished with woodflakes. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (Rat and Mouse Expanded Diet No.1, Special Diets Services Limited, Witham, Essex, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminants that would reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items: wooden chew blocks (B&K Universal Ltd, Hull, UK) and cardboard fun tunnels (Datesand Ltd, Cheshire, UK) or suitable alternatives. These items were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Specific Gravity	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats	
				Male	Female
2000	0.945	-	2.12	-	3
200	-	20	10	-	3
200	-	20	10	3	-

All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe. The volume administered to each animal was calculated according to the fasted bodyweight at the time of dosing. Treatment of animals was sequential. Sufficient time was allowed between each sex and each dose level to confirm the survival of the previously dosed animals.

The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing and subsequently once daily for up to fourteen days.

Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment or at death.

At the end of the observation period the surviving animals were killed by intravenous injection of sodium pentobarbitone. All animals were subjected to gross necropsy. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

3.3 Evaluation of Data

Data evaluations included the relationship, if any, between the exposure of the animal to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight changes, mortality and any other toxicological effects.

Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD_{50}) of the test material was made as shown in the schematic diagram in Appendix 1.

The results were evaluated according to Commission Directive 93/21/EEC for classification and labelling of dangerous substances and preparations.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Mortality Data

Individual mortality data are given in Table 1.

All females treated at a dose level of 2000 mg/kg were found dead thirty minutes or one hour after dosing. No deaths were noted in males or females treated at a dose level of 200 mg/kg.

5.2 Clinical Observations

Individual clinical observations are given in Tables 2 and 3.

Signs of systemic toxicity noted at 30 minutes after dosing in one female treated at a dose level of 2000 mg/kg were clonic convulsions, exophthalmos, decreased respiratory rate and laboured and noisy respiration. No clinical observations were conducted for the other two females due to deaths prior to the first observation period.

Signs of systemic toxicity noted in animals treated at a dose level of 200 mg/kg were exophthalmos, lethargy and occasional body tremors during the day of dosing. Hunched posture was also noted until two days after dosing.

5.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Tables 4 and 5.

The surviving animals showed expected gains in bodyweight over the study period.

5.4 Necropsy

Individual necropsy findings are given in Tables 6 and 7.

Abnormalities noted at necropsy of the females treated at a dose level of 2000 mg/kg that died during the study were haemorrhagic lungs, dark liver, dark kidneys and slight haemorrhage of the small intestine. No abnormalities were noted at necropsy of animals treated at a dose level of 200 mg/kg that were killed at the end of the study.

6. CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test material in the Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rat was estimated to be in the range of 300 - 500 mg/kg bodyweight.

The test material was classified as HARMFUL and the symbol "Xn" and risk phrase R 22 "HARMFUL IF SWALLOWED" are required according to EU labelling regulations Commission Directive 93/21/EEC.

KEY TO CLINICAL OBSERVATIONS

B	=	exophthalmos
Cc	=	clonic convulsions
H	=	hunched posture
L	=	lethargy
Rd	=	decreased respiratory rate
Rl	=	laboured respiration
Rn	=	noisy respiration
To	=	occasional body tremors
0	=	no signs of systemic toxicity
X	=	animal dead

Table 1 Mortality Data

Dose Level mg kg	Sex	Number of Animals Treated	Deaths During Day of Dosing (Hour)				Deaths During Period After Dosing (Days)								Deaths
			½	1	2	4	1	2	3	4	5	6	7	8-14	
2000	Female	3	2	1	-	-	-	-	-	-	-	-	-	-	3/3
200	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	Male	3	0	0	0	0	0	0	0	0	0	0	0	0	0/3

- = All animals dead

Table 2 Individual Clinical Observations - 2000 mg/kg

Animal Number and Sex	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)													
	½	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1-0 Female	X																	
1-1 Female	CcRd RlB Rn	X																
1-2 Female	X																	

Table 3 Individual Clinical Observations - 200 mg/kg

Animal Number and Sex	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)													
	½	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2-0 Female	HLB To	HLB To	HLB To	HLB To	H	H	0	0	0	0	0	0	0	0	0	0	0	0
2-1 Female	HLB To	HLB To	HLB To	HLB To	H	H	0	0	0	0	0	0	0	0	0	0	0	0
2-2 Female	HLB To	HLB To	HLB To	HLB To	H	H	0	0	0	0	0	0	0	0	0	0	0	0
3-0 Male	To	HLTo	HLTo	HL	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3-1 Male	To	HLTo	HLTo	HL	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3-2 Male	To	HLTo	HLTo	HL	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4 **Individual Bodyweights and Weekly Bodyweight Changes - 2000 mg/kg**

Animal Number and Sex	Bodyweight (g) at Day			At Death	Bodyweight Gain (g) During Week	
	0	7	14		1	2
1-0 Female	223	-	-	223	-	-
1-1 Female	212	-	-	212	-	-
1-2 Female	242	-	-	242	-	-

- Animal dead

Table 5 Individual Bodyweights and Weekly Bodyweight Changes - 200 mg/kg

Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
	0	7	14	1	2
2-0 Female	221	248	264	27	16
2-1 Female	225	240	260	15	20
2-2 Female	228	237	250	9	13
3-0 Male	200	266	322	66	56
3-1 Male	207	272	318	65	46
3-2 Male	215	291	321	76	30

Table 6 **Individual Necropsy Findings - 2000 mg/kg**

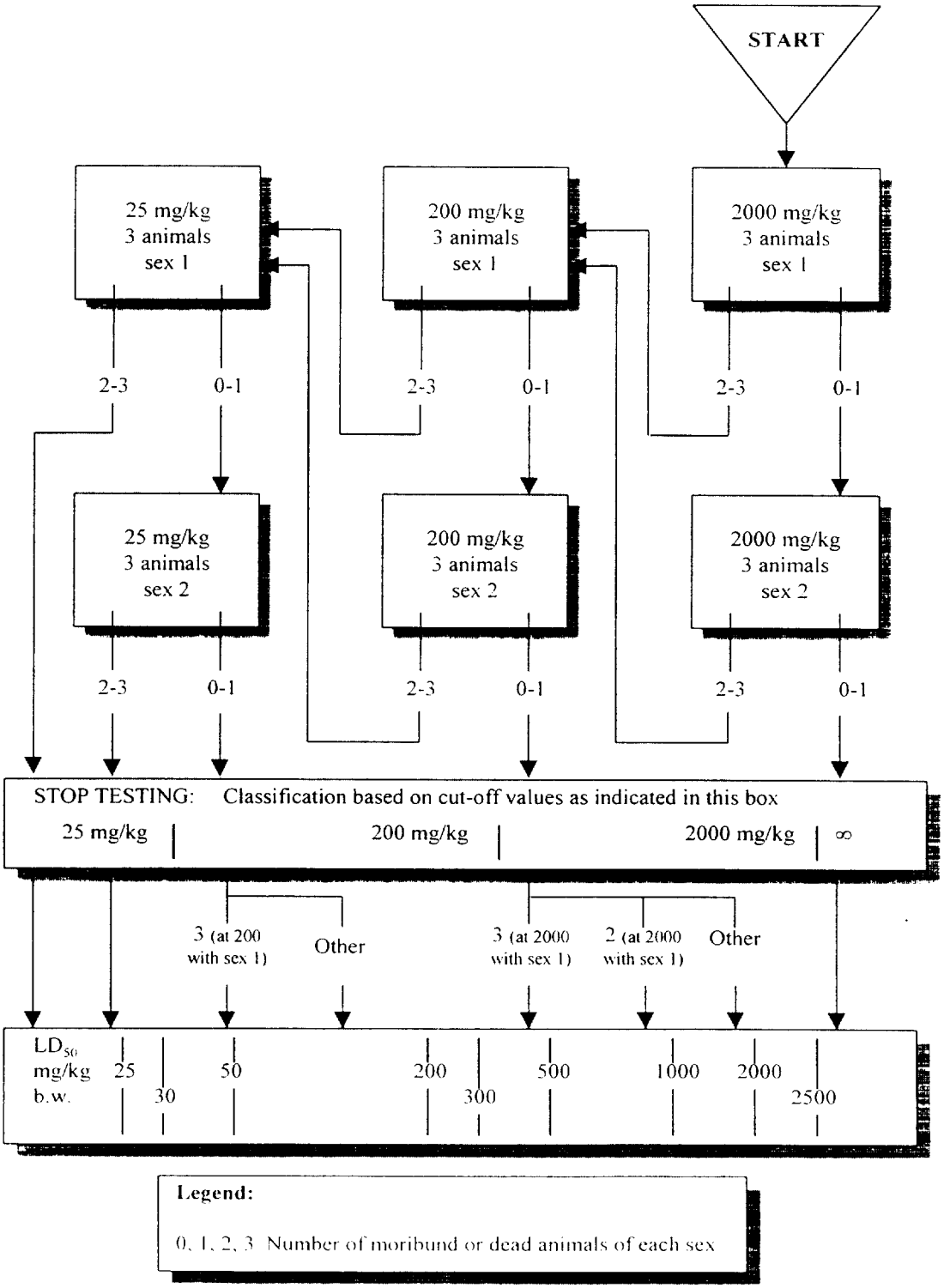
Animal Number and Sex	Macroscopic Observations
1-0 Female	Lungs: haemorrhagic Liver: dark Kidneys: dark Small intestine: slight haemorrhage
1-1 Female	Lungs: haemorrhagic Liver: dark Kidneys: dark
1-2 Female	Lungs: haemorrhagic Liver: dark Kidneys: dark

Table 7 **Individual Necropsy Findings - 200 mg/kg**

Animal Number and Sex	Macroscopic Observations
2-0 Female	No abnormalities detected
2-1 Female	No abnormalities detected
2-2 Female	No abnormalities detected
3-0 Male	No abnormalities detected
3-1 Male	No abnormalities detected
3-2 Male	No abnormalities detected

ACUTE ORAL TOXICITY IN THE RAT
– ACUTE TOXIC CLASS METHOD

Appendix 1 Test Procedure with a Starting Dose of 2000 mg/kg Bodyweight



Appendix 2 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Ltd
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE

Analytical Chemistry
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Tests
Toxicology

DATE OF INSPECTION
28 February 2000

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

Appendix 3 Copy of Protocol

SafePharm Laboratories

PROTOCOL

TEST MATERIAL	:	
STUDY TYPE	:	Acute Oral Toxicity Study in the Rat - Acute Toxic Class Method
TEST METHOD	:	512.06
PROJECT NUMBER	:	1014/132
PROPOSED START DATE	:	End of February 2001
PROPOSED COMPLETION DATE	:	End of March 2001
TARGET (DRAFT) REPORT DATE	:	Early May 2001
SPONSOR	:	

SPECIAL CONDITIONS : The animals will be sacrificied using intravenous injection of sodium pentabarbitone.

This study is compliant with the following toxicology guidelines:

- OECD Test Guideline 423, 1996
- Method B1 tris Acute Toxicity (Oral) of Commission Directive 96/54/EC

AUTHORISED BY: ..... DATE: 08 FEB 2001

P Brunt HNC
STUDY DIRECTOR

APPROVED FOR SPONSOR BY: ..... DATE: March 6, 2001

ACUTE ORAL TOXICITY STUDY IN THE RAT - ACUTE TOXIC CLASS METHOD

1. INTRODUCTION AND OBJECTIVES

To assess the toxicity of the test material following a single oral dose to the rat. The results of the study are believed to be of value in predicting the likely toxicity in man by the oral route and provide information for hazard classification purposes.

The method uses defined starting doses and is not intended to allow the calculation of a precise LD_{50} but does allow for the determination of a range of exposures where lethality is expected since death of a proportion of the animals is still a major end-point of the test.

The work described will be performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

2. TEST FACILITY

Safepharm Laboratories Ltd.
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD

3. ANIMALS

Specification: Sprague-Dawley Crl:CD® (SD) IGS BR strain rats obtained from Charles River (UK) Limited, Margate, Kent. Females will be nulliparous and non-pregnant. At the start of the study animals will be aged eight to twelve weeks and will weigh at least 200g. The weight variation will not exceed ± 20% of the mean weight for each sex.

Justification: Preferred species of choice as historically used for safety evaluation studies and specified by appropriate regulatory authorities.

4. ANIMAL HUSBANDRY

Environment:

Target temperature:	19 to 25°C
Target humidity:	30 to 70%
Lighting:	twelve hours of continuous artificial light in each twenty-four hour period
Ventilation:	at least fifteen changes per hour

Housing: Groups of up to three by sex in suspended polypropylene cages furnished with softwood woodflakes and fitted with stainless steel mesh lids. Results of routine analysis of the woodflakes are made available to Safepharm Laboratories Ltd.

Diet and Water: Rat and Mouse SQC Expanded Diet No.1 (Special Diets Services Limited, Witham, Essex, UK), and tap water *ad libitum*. Food removed overnight prior to dosing and returned approximately three to four hours after dosing.

The diet and water are routinely analysed and are considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.

5. PRE-TEST PROCEDURES

Acclimatisation Period: At least five days.

Identification: Each animal, selected at random, will be uniquely identified within the study by indelible ink markings on the tail. A colour-coded cage card will be prepared with details of test material, project number, dose level, sex, number of animals, route of administration and initials of the Study Director.

6. TEST MATERIAL AND EXPERIMENTAL PREPARATION

Supply: Supplied by Sponsor with details of hazardous properties if known. Data relating to the identification, purity and stability of the test material will be the responsibility of the Sponsor.

Storage: Room temperature in the dark unless otherwise specified by the Sponsor.

Preparation: Wherever possible, liquids will be administered as supplied at a variable dose volume. Dilutions of liquid test materials may be made where the toxicity of the test material is high and dose volume would be too low to ensure accurate administration of the undiluted sample.

Viscous liquids and solids will be formulated in a suitable vehicle. When formulation of the test material is necessary, preparation of an aqueous solution will firstly be attempted. If preparation of an aqueous solution is not possible, preparation of a solution in vegetable oil (eg arachis oil) will be considered, followed by solution in other suitable vehicles, or in suspension.

Analysis: All formulations will be used within two hours of preparation and will be assumed to be stable for this period unless specified otherwise by the Sponsor. The concentration and homogeneity of the formulations will not be determined by analysis.

7. TEST MATERIAL ADMINISTRATION

Administration: Once only by gavage. If a single dose is not possible, the dose may be given in small fractions over a period not exceeding twenty-four hours.

Dose Volumes: The dose volume will be a maximum of 20 ml/kg for aqueous preparations and a maximum of 10 ml/kg for other vehicles.

Absorption: The absorption of the test material will not be determined.

8. STUDY DESIGN

Number of Animals: Three animals of one sex are used for each step. Either sex can be used in the initial step.

Dose Levels: The dose level to be used as the starting dose will be selected from one of the three fixed levels: 25, 200 or 2000 mg/kg bodyweight. The starting dose level will be that which is most likely to produce

mortality in at least some of the dosed animals. One of the flow charts of the procedures described in the Annex will be used depending on the starting dose.

For selecting the sex and the starting dose, all available information will be used. When the information suggests that mortality is unlikely at the highest dose level (2000 mg/kg bodyweight), then a limit test will be conducted with three animals of each sex. In the absence of any relevant toxicity data 200 mg/kg will be used in the first instance.

It may be necessary to achieve a further refinement of classification than is possible after conducting the test with the three fixed dose levels of 25, 200 and 2000 mg/kg bodyweight. Further testing at additional fixed dose levels of 5, 50 or 500 mg/kg bodyweight may be considered.

The time interval between treatment groups will be determined by the onset, duration and severity of toxic signs. Treatment of animals of the other sex, or at the next dose, will be delayed until survival of the previously dosed animals is confirmed.

9. OBSERVATIONS

Morbidity/Mortality Inspection: Twice daily, early and late, during normal working days, once daily at weekends and public holidays. The time of death will be recorded as precisely as possible.

Clinical Observations: Half an hour and 1, 2 and 4 hours after dosing, then at least once daily for 14 days. The observation period may be extended if signs of toxicity are persistent at Day 14. Types of visually observed signs of toxicity, the time at which signs of toxicity are noted, and the time of death will be recorded for all individual animals.

Observations will include changes in the skin and fur, eyes and mucous membranes, and respiratory, circulatory, autonomic and central nervous system, and somatomotoractivity and behaviour pattern. Particular attention will be directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

Throughout the study animals may be humanely killed *in extremis* in order to prevent pain or suffering.

Bodyweights: Recorded on Day 0 (prior to dosing), Day 7 and 14, or at death.

Necropsy and Pathology: A gross necropsy will be performed on all animals dying during the study and on all survivors killed by cervical dislocation on Day 14. Any macroscopic abnormalities will be recorded.

At the discretion of the Study Director, organs with macroscopic abnormalities may be preserved in buffered formalin. Histopathological examination will only be performed after consultation with and at extra cost to the Sponsor.

10. EVALUATION OF DATA

Data evaluations will include an assessment of the number of animals displaying signs of treatment-related toxicity, the number of animals found dead during the study or killed for humane reasons, a description of the toxic effects and the time course of any toxic effects. Animals which are humanely killed due to test material-related pain or distress will be regarded as treatment-related deaths.

11. QUALITY ASSURANCE

The final report will be audited by Safepharm Quality Assurance Unit. This type of study is subject to process-based QA inspections designed to encompass the major phases once per month.

12. PROTOCOL AMENDMENTS

Amendments to this protocol will be made only by completion of an amendment to protocol form authorised by the Study Director.

13. FINAL REPORT

The final report will include, as a minimum, the following information:

Details of test material and vehicle (if appropriate)
Justification for choice of vehicle, if other than water
Species, strain, supplier of animals

Number, age and sex of animals
Environmental conditions and animal diet
Test conditions
Dose levels (with vehicle if used, and concentrations)
Rationale for the selection of the starting dose
Tabulation of response data by sex, dose level and time (ie animals showing signs of toxicity including mortality, nature, severity and duration of effects)
Clinical observations and bodyweight data
Reasons and criteria used for humane killing of animals
Necropsy findings
Discussion of results
Interpretation of results (including appropriate classification of toxicity and risk phrase if required)

14. ARCHIVE

Unless otherwise instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for a period of five years after which instructions will be sought as to further retention or disposal. Further retention or return of the data will be charged at extra cost to the Sponsor.

ANNEX I

REMARKS

1. As indicated in Section 8, the starting dose should be the one which is likely to produce mortality in at least some of the dosed animals. Information that could be used to select the starting dose include:

   - data on physical chemical properties
   - structure-activity relationships
   - all data from other toxicity tests; and
   - anticipated use of the test substance

2. For each starting dose, the respective testing schemes as included in this annex outline the procedure to be followed. Depending on the number of humanely killed or dead animals, the test procedure follows the indicated arrows.

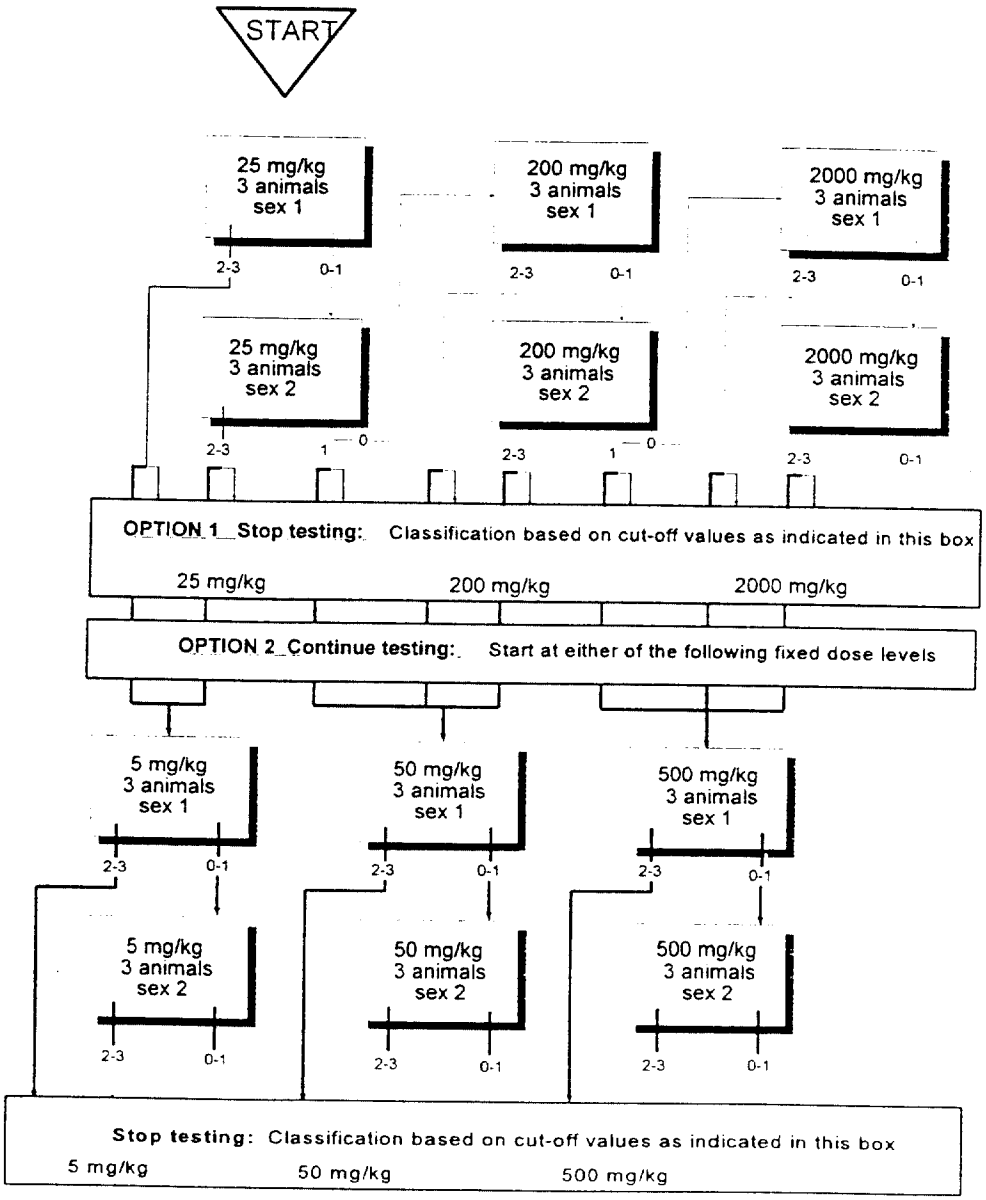
3. When at a starting dose of 25 or 200 mg/kg bodyweight only one animal of the second sex dies, this would normally lead to no further testing. However, when no toxic signs are observed in the other five animals during autopsy, consideration should be given to the possibility that mortality may not have been compound related. In such a case, the test should be continued with dosing at the next higher level.

4. When at dose of 2000 mg/kg bodyweight, one animal per sex dies, the LD_{50} value is expected to exceed 2000 mg/kg bodyweight. However, because this is a "borderline" result, the response of the remaining two animals per sex should be carefully considered and the occurrence of distinct, marked toxic signs in these animals may well lead to classification corresponding to an LD_{50} value of 2000 mg/kg bodyweight or less or would justify further testing at this same level.

5. The procedure allows for testing at three additional fixed doses (option 2). This option could either be used to select an alternative dose at a given decision point, or for further testing after having completed the actual test (option 1). The option 1 test procedure is indicated with thin arrows, whereas for the option 2 test procedure, bold arrows are used.

ANNEX Ia

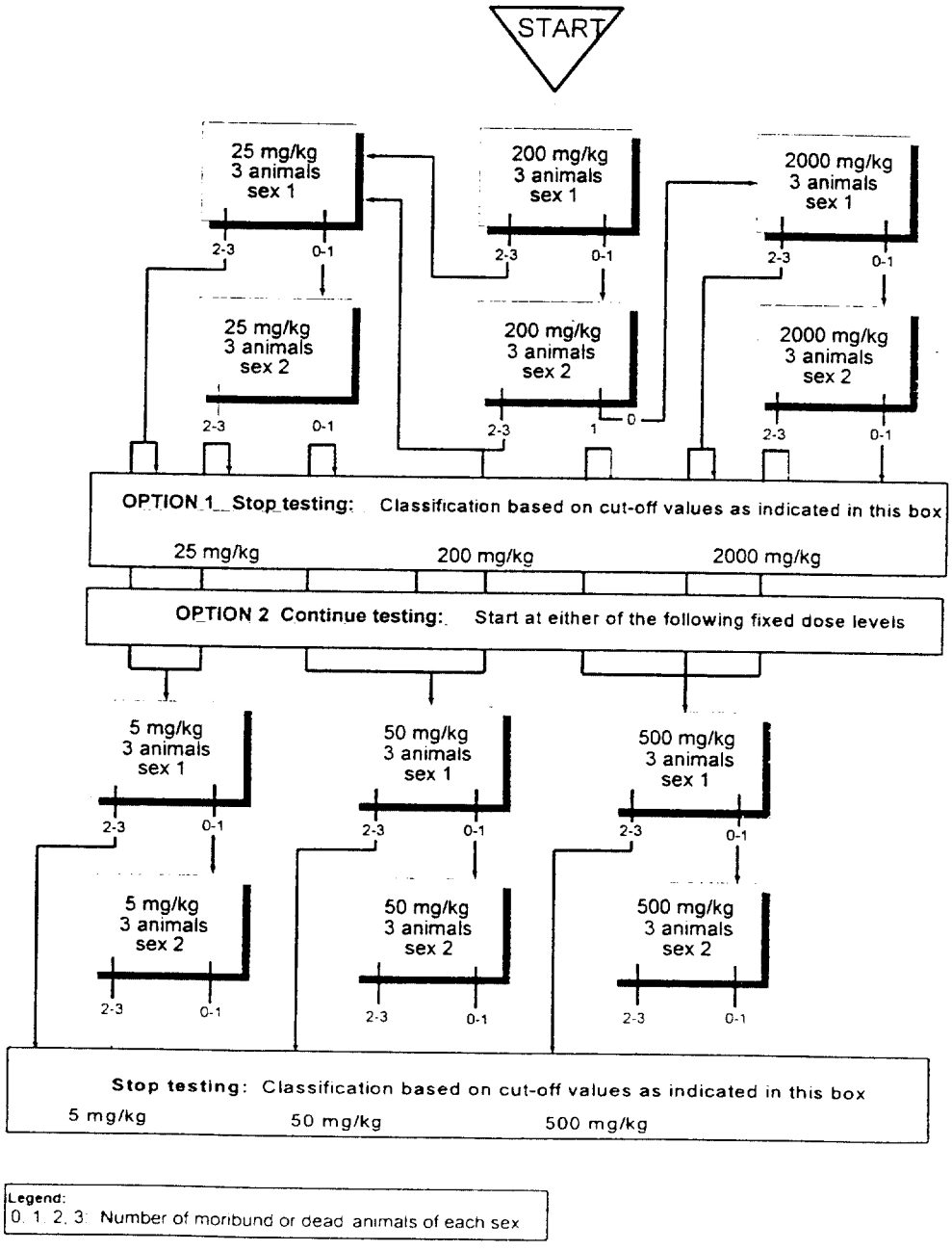
TEST PROCEDURE WITH A STARTING DOSE OF 25 mg/kg BODYWEIGHT



Legend:
0, 1, 2, 3 Number of moribund or dead animals of each sex

ANNEX 1b

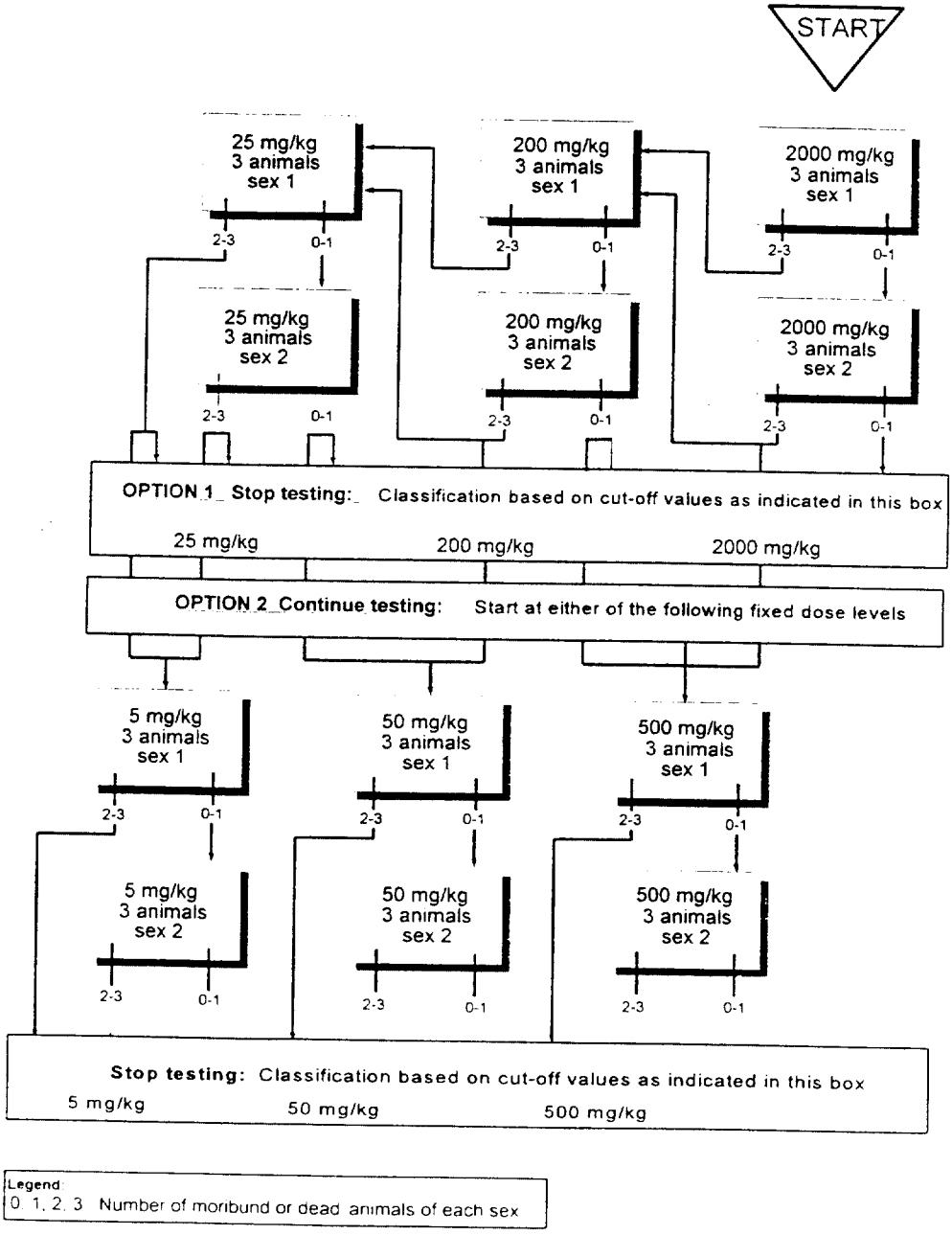
TEST PROCEDURE WITH A STARTING DOSE OF 200 mg/kg BODYWEIGHT



Legend:
0, 1, 2, 3: Number of moribund or dead animals of each sex

ANNEX Ic

TEST PROCEDURE WITH A STARTING DOSE OF 2000 mg/kg BODYWEIGHT

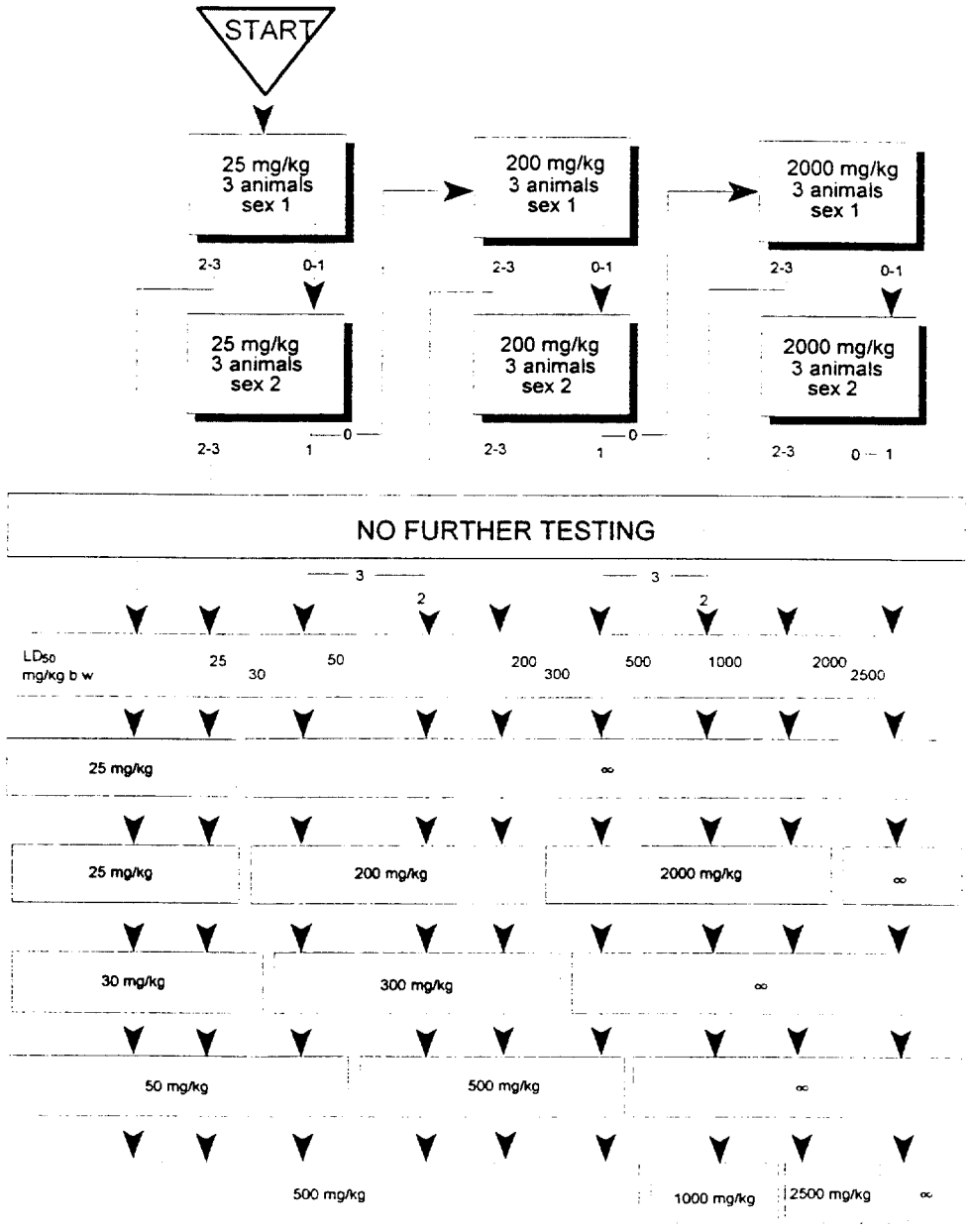


ANNEX 2

INTERPRETATION OF RESULTS BASED ON OPTION 1 TESTING

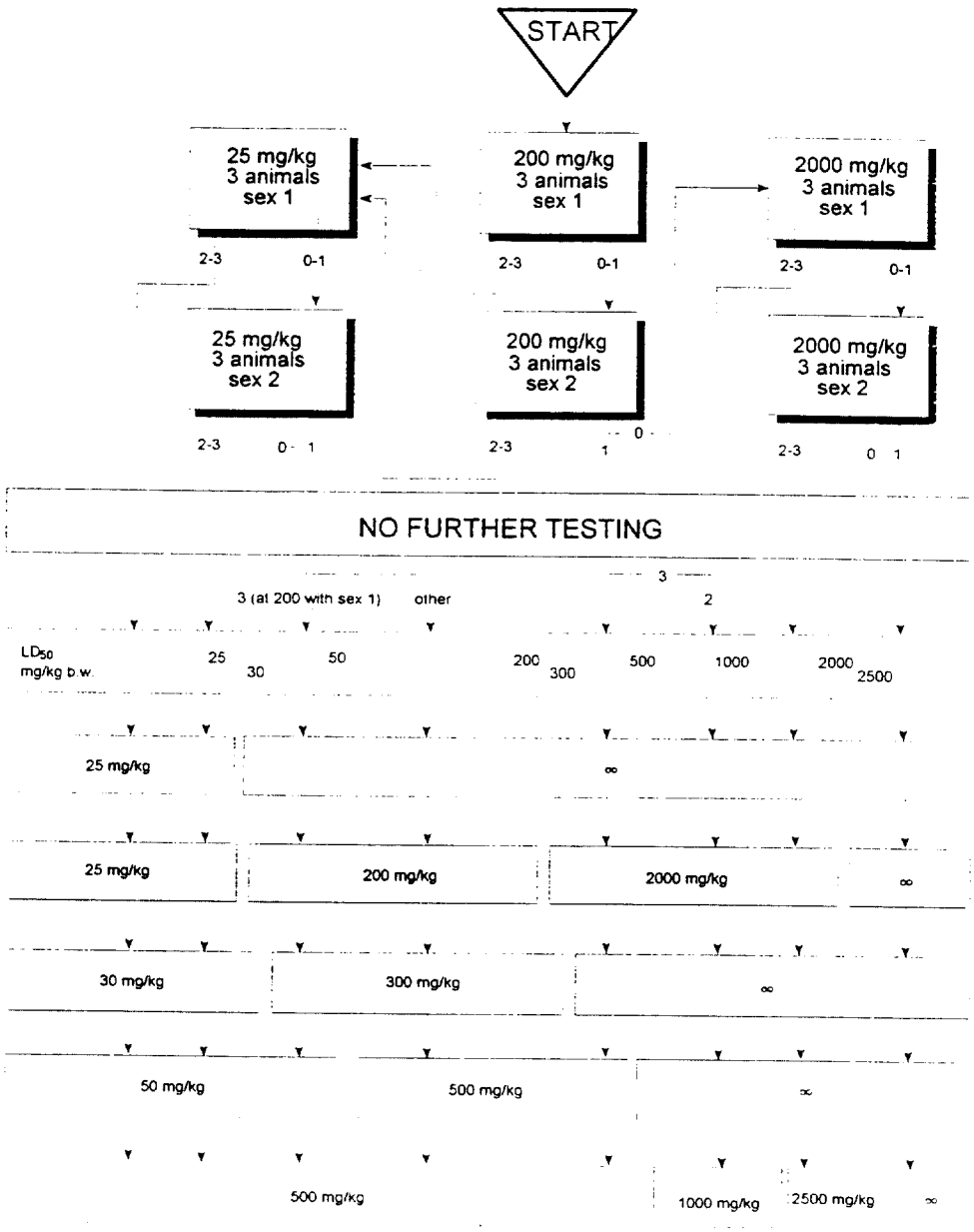
The grey boxes below the "no further testing" box in the schemes of this annex, represent cut off values for classification. Following the test procedure as outlined in Option 1, the appropriate arrow should be followed further downwards, until it reaches the grey box of concern.

ANNEX 2a

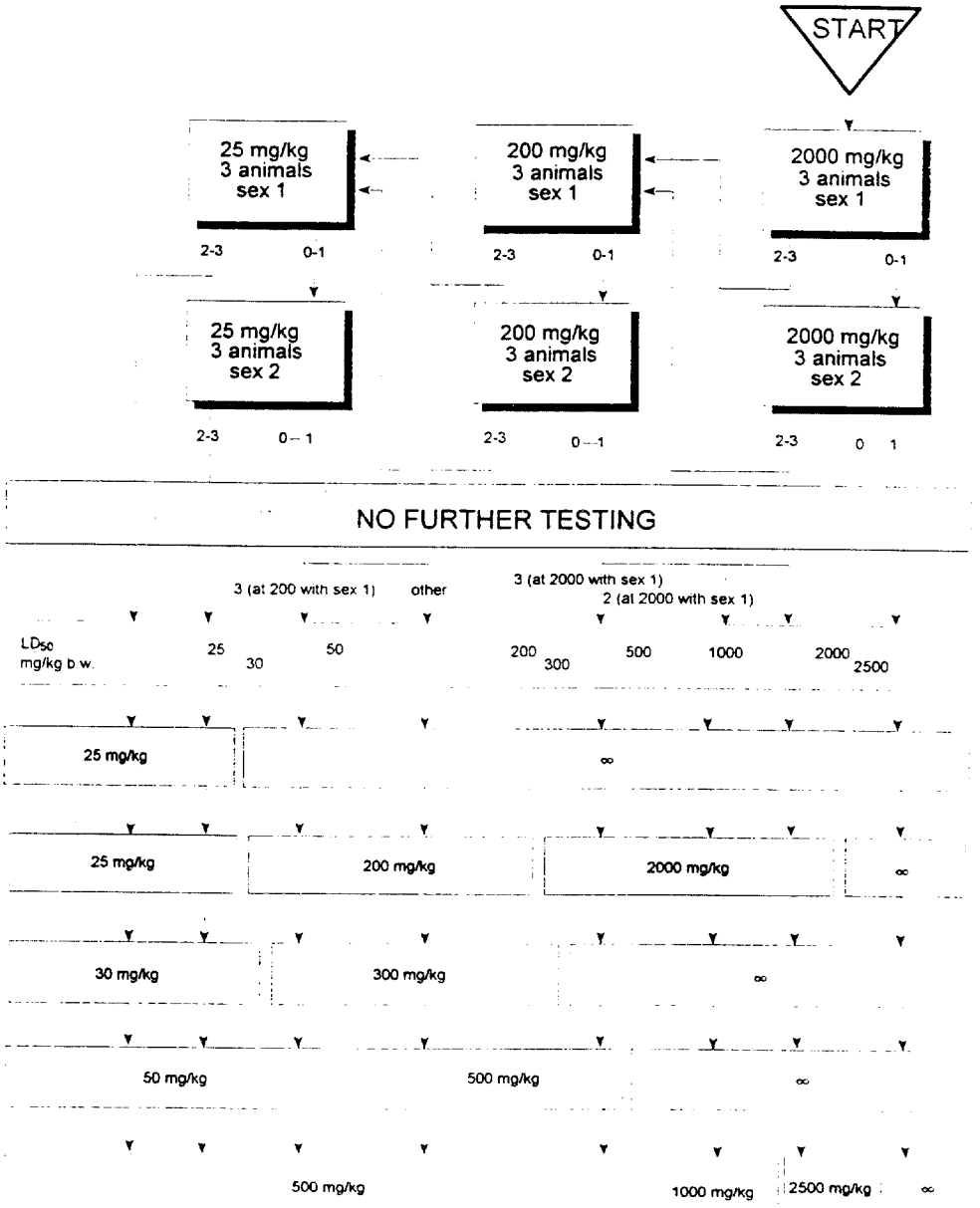


Legend
0, 1, 2, 3 : Number of moribund or dead animals of each sex

ANNEX 2b


START
25 mg/kg 3 animals sex 1
200 mg/kg 3 animals sex 1
2000 mg/kg 3 animals sex 1
2-3
0-1
25 mg/kg 3 animals sex 2
200 mg/kg 3 animals sex 2
2000 mg/kg 3 animals sex 2
NO FURTHER TESTING
3 (at 200 with sex 1)
other
3
2
LD50 mg/kg b.w.
25
30
50
200
300
500
1000
2000
2500
25 mg/kg
∞
200 mg/kg
2000 mg/kg
30 mg/kg
300 mg/kg
50 mg/kg
500 mg/kg
1000 mg/kg
2500 mg/kg

ANNEX 2c


START
25 mg/kg 3 animals sex 1
200 mg/kg 3 animals sex 1
2000 mg/kg 3 animals sex 1
2-3
0-1
25 mg/kg 3 animals sex 2
200 mg/kg 3 animals sex 2
2000 mg/kg 3 animals sex 2
NO FURTHER TESTING
3 (at 200 with sex 1)
other
3 (at 2000 with sex 1)
2 (at 2000 with sex 1)
LD50 mg/kg b.w.
25
30
50
200
300
500
1000
2000
2500
25 mg/kg
∞
25 mg/kg
200 mg/kg
2000 mg/kg
∞
30 mg/kg
300 mg/kg
∞
50 mg/kg
500 mg/kg
∞
500 mg/kg
1000 mg/kg
2500 mg/kg
∞